Evaluations on Efficacy of a Manual Therapy utilizing Imaging Analysis

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博士論文

Evaluations on Efficacy of a Manual Therapy utilizing Imaging Analysis

（用手療法の有用性に関する画像解析を用いた科学的検討）

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ABSTRACT

Objective: Chiropractic spinal manipulation (CSM) is known as an alternative treatment mainly for back pain. It is included in one of the alternative cares of medicine by world health organization (WHO) and is presently legislated over 20 countries in the world. Although there are around 8,000 chiropractic practitioners in Japan, there is no official licensure system and official educational system unlike other developing countries. Despite clinical evidences for benefits of CSM and apparent wide usage of it, physiological mechanisms underlying the effects of CSM are not clearly known. The aim of the present study is to investigate the effects of CSM on brain responses in terms of cerebral glucose metabolic changes measured by FDG-PET.

Methods: Twelve male volunteers were recruited in the present study. Brain PET scanning was performed twice on each subject, the resting condition and the treatment condition, and PET images were compared between those two conditions. Questionnaires were used for subjective evaluations. Visual analogue scale (VAS) was rated by subjects before and after CSM for an evaluation of subjective pain sensation. Muscle tone at the upper trapezius muscle was measured bilaterally in resting and treatment conditions to evaluate a peripheral effect, and salivary amylase was also measured in two conditions to evaluate the autonomic response. The electrocardiogram (ECG) monitoring was also performed in resting and treatment conditions to analyze heart rate variability (HRV) for evaluating autonomic response. Additionally, correlation analyses were performed between PET images and the VAS scores of subjective pain sensation.

Results: In the present study, significant differences were observed on some brain areas. Increased glucose metabolism was observed in the inferior prefrontal cortex, anterior cingulated cortex, and middle temporal gyrus whereas decreased glucose metabolism was observed in the cerebellar vermis and the visual association cortex on the PET analysis in the treatment condition ($p<0.001$). The scores of the questionnaire which indicate stress level was lower in the treatment condition. The comparisons of VAS scores revealed significantly lower values after CSM. Measurements of cervical muscle tone and salivary amylase also indicated reduced values in the treatment condition. The correlation analysis indicated a positive correlation between the regional cerebral glucose metabolism in the orbitofrontal cortex and in the caudate nucleus and VAS scores of subjective pain sensation.

Conclusion: Results of the present study might indicate CSM procedure affects on the regional cerebral metabolism related to relaxation and pain reduction which are associated with changes of the autonomic function. Therefore, it is possible to state that a single CSM treatment affects the central nervous system.

Keywords: Chiropractic, spinal manipulation, cervical pain, cerebral metabolism, positron emission tomography (PET)

BACKGROUND AND INTRODUCTION

ñChiropracticò is one of the manipulative therapies initiated by Daniel David Palmer in 1895 in the United States, and its purpose has been thought to be ñimproving neurophysiological functions that lead to enhancing the ability of natural healing of the body by adjusting dysfunction of the joint called subluxation in the spine, pelvis, and extremity[1].ò Currently, chiropractic is legislated in the U.S. and in over 20 other countries of the world. World health organization (WHO) has stated that all modalities of treatment in the modern and traditional systems of medicine need to play a role in accordance with the salutary effects afforded by the different systems of medicine for equitable access to health services[2]. WHO has been promoting chiropractic as one of the most prevailing and prominent treatment options[2]. Based on such situation, for instance, in the U.S., there is a 6-academic-year educational system to obtain the Doctor of Chiropractic (D.C.) degree. Currently, approximately 60,000 D.C.s are practicing in the U.S. In contrast, there is no official 6-year-long educational system to obtain the D.C. degree in Japan. According to a personal contact to the president of a Japanese science news company, which publishes a chiropractic journal, there are approximately 8,000 chiropractic therapists (chiropractors) in Japan, but only around 100 hold D.C. degrees from the U.S. chiropractic schools (less than 2%).

In the late 1980s, stimulated by the increase in the number of chiropractors and prevailing of this treatment in the population in Japan, the Ministry of Welfare performed a study on chiropractic. In that study, Miura et al. stated that efficacy of chiropractic treatment includes 1) reduction of sensitiveness, 2) rise in the threshold of pain perception, and 3) relief of sympathetic excitation; however, this statement has been thought to be only an empirical one by chiropractic practitioners not based on the scientific data[3]. They also indicated that, due to lack of scientific data regarding the efficacy of chiropractic, further scientific studies on chiropractic should be done in order to conduct proper medical evaluations, as described in so called "Miura Report" published in 1991[3]. For the last 30 years, scientific evaluation regarding the efficacy of chiropractic has been paid little attention in spite of various clinical evidences for benefits of the treatment. Nowadays, chiropractic has been getting more and more popular in many countries except for Japan, and encouraged by WHO as one of the most effective alternative cares. Therefore, based on such background, the main aim of this study has been to demonstrate scientific data regarding the efficacy of chiropractic treatment using the modern medical imaging technique.

Chiropractic spinal manipulation (CSM) is considered as one of the main treatment techniques for neuro-muscular-skeletal problems, and its main complaints of the patient are neck pain, back pain and low back pain. For more than 100 years, chiropractors have asserted that overall health can be improved through the use of spinal manipulative therapy[4) 5) 6) 7) 8)]. Despite clinical evidences for benefits of the spinal manipulation and apparent wide usage of it, physiological mechanisms underlying the effects of CSM are not clearly known[9)].

Many studies on CSM have been performed in the world[4) 9) 10)]. Main components of these studies are long term follow-up studies or case reports for clinical efficacy on CSM, and it seems no study has been done on effectiveness of a certain type of CSM techniques utilizing sophisticated medical imaging such as positron emission tomography (PET).

In addition, the autonomic nervous system is thought to be one of the areas influenced by CSM. A study documented that the autonomic nervous system has been often invoked in constructing the mechanisms to account for the effect due to spinal dysfunction[9)]. To the best of our knowledge; however, no study has been performed on the regional cerebral metabolic changes related to autonomic responses from CSM utilizing functional neuroimaging techniques. Functional neuroimaging techniques are powerful tools when it comes to the investigation of neuronal activity in the human brain[10)]. PET has been typically utilized for measuring the regional cerebral blood flow or regional cerebral metabolic rate, using radio-labeled tracers, which are usually injected intravenously or continuously inhaled by patients or research participants[10) 11) 12) 13)]. This technique might be able to elucidate the neural mechanism of relaxation effects due to CSM treatment. In fact, studies on other alternative cares such as acupuncture and aroma therapy demonstrated therapeutic effects on the autonomic nervous system[14) 15)]. Fang et al. demonstrated, using a PET-CT, that the acupuncture treatment for functional dyspepsia patients showed changes of regional cerebral metabolism in some regions associated with gastric perceptions[14)]. Duan et al. also demonstrated, in a PET study, that lavender aromatic treatment induced regional cerebral metabolic changes associated with relaxation indicated by heart rate variability (HRV) measurement[15].

Thus, the aim of the present study is to investigate effects of a single CSM on brain responses in terms of the cerebral glucose metabolic changes measured by PET with ^{18}F-fluorodeoxyglucose (FDG). Additionally, this study discusses relationship of the changes of autonomic functions and pain intensity to the regional cerebral glucose metabolic activities before and after CSM. It may be possible to demonstrate indirect and central effects of a single CSM on the central nervous system.

Regional Cerebral Metabolic Changes in Patients with Cervical Pain after Chiropractic Spinal Manipulation: ^{18}F- FDG-PET Analysis

INTRODUCTION

Chiropractic spinal manipulation (CSM) has been considered as an alternative care for neuro-muscular-skeletal problems, and its main complaints of the patient are neck pain, back pain and low back pain. It is also stated that the autonomic nervous system has often been involved in the effect of spinal dysfunction[9]. Previous studies documented a potential relationship between the vertebral misalignment and the function of autonomic nervous system[16,17,18,19,20]. According to a study by Budgel, where sympathetic mediation has been significant, it has been possible to demonstrate the existence of spinal reflex centers and, to some degree, a measure of segmental organization[16]. However, physiological mechanism underlying effects of CSM to the autonomic nervous system is not clearly known[9]. Although studies on relationship between spinal misalignment and the autonomic nervous functions have been performed, no chiropractic study has been performed in terms of the regional cerebral metabolism measured utilizing positron emission tomography (PET).

Functional neuroimaging techniques are powerful tools when it comes to investigation of neuronal activity in the human brain[10]. PET is typically utilized for measuring regional cerebral blood flow or regional cerebral metabolic rate, using radio-labeled pharmaceuticals, which are usually injected intravenously or continuously inhaled by patients or research participants[10,11,12,13]. Recently, researchers stated that PET is a modern diagnostic technique which enables visualization and measurement of brain morphology and function[21].

In the research setting, perfusion PET with radioactive water has traditionally been used in activation studies, and for this purpose functional MRI (fMRI) has to large extent replaced PET because of the problems of radiation to the patient, spatial resolution and operating cost per investigation[10) 13) 22)]. However, FDG-PET is presently thought to be the adoption of metabolic imaging into intraoperative and minimally invasive setting[23)]. FDG, an analogue of glucose, is trapped metabolically in active cells after being administered into the body, and can be substantially used for evaluating physiological and biomechanical functions in vivo[24)].

There is a study that evaluated the regional cerebral metabolism after CSM. In that study, single photon emission computed tomography (SPECT) with technetium ^{99m}Tc-ethyl cysteinate dimer (^{99m}Tc-ECD) was utilized[25)]. The study documented that hypoperfusion in the anterior lobe of the left cerebellum was observed after a manual type of the cervical manipulation[25)]. The authors concluded that the results of the study could explain why certain people experience headache, dizziness, or nausea after CSM as an adverse reaction of the manual cervical manipulation[25)]. It is thought that further functional neuroimaging investigations have been needed on efficacy of CSM since the study was performed by use of a manual rotatory cervical adjustment which might cause the adverse reaction on the patient. Thus, the aim of the present study is to examine the effects of CSM in the brain through measuring cerebral metabolic changes by using FDG-PET.

METHODS

Subjects and Materials

Twelve male volunteers who had cervical pain at the time of this experiment, age of 21 to 40 (mean age +/- S.D.: 28+/-6.8), were recruited at Tohoku University for the present study after obtaining their written informed consent. Subjects with no experience of CSM or who had not undergone any type of treatments for their cervical pain at least one month prior to the experiment were recruited for the present study. Duration of the subjectōs cervical pain was 3 weeks to 10years (mean duration +/- S.D.: 35+/-36.8 months), and causes of the cervical pain included a use of the personal computer in 5 subjects, a gradual onset (unknown cause) in 6 subjects, and a golf injury in a subject. All subjects were assigned for MRI examination of their cervical region before inclusion in this study, and subjects with pathological problems such as tumors, disc herniations, and significant disc degenerations were excluded. Medical screening was performed on the subjects to exclude disorders and medication that might affect brain function or perfusion. It was also confirmed that all subjects had not experienced nerve root symptoms such as numbness of the upper extremity by the medical screening. In addition, some orthopedic examinations were performed on all subjects before inclusion of this study; cervical compression tests including foraminal compression test, Jacksonōs teast, extension compression test and flexion compression test were performed to exclude the cervical nerve root problem, and Maigneōs test was performed to exclude arctation of the vertebral artery which is a contraindication to the cervical manipulation. Female subjects were not included because of the risk of radiation exposure. The present study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine.

Brain PET scanning was performed twice on each subject with administration of FDG injected through the left cubital vein (mean +/- SD: 47.0+/-8.9 MBq) 30 minutes prior to the scanning. Subjects were asked to sit and to get relaxed with their eyes closed for 30 minutes before scanning. One scan was performed after a 20-minute-long resting phase (resting condition) as a baseline, and the other was performed after CSM (treatment condition) with an interval of 7 to 42 days (22.4+/-12.5 days) between the two scans. The order of 2 scans, for the resting and treatment conditions, was counter-balanced across the subjects in order to eliminate an order effect. CSM took around 20 minutes for each subject including diagnostic procedures. Following the treatment and the rest, each subject was scanned using a PET scanner, SET 2400W (Shimadzu Inc., Kyoto, Japan). The PET scanning covered entire brain and took 10 minutes, and a 5-minute-long transmission scan followed for tissue attenuation correction. Electrocardiogram (ECG) recording was performed on all subjects in resting and treatment conditions following the FDG administration for 10 minutes to evaluate heart rate variability (HRV). Two chest electrodes, Magnerode, TE-18 (Fukuda Denshi Co., LTD., Tokyo, Japan) were attached on subjectsôchest, over manubrium of the sternum and the left fifth intercostal space, about 1 cm medial to the mid-clavicular line for ECG recording.

Subjects were requested to keep a relaxed-respiration during ECG monitoring. ECG recording was obtained by using polar 810i (Polar Electro Oy, Finland). Comparisons of HRV parameters (normalized low frequency: nLF and normalized high frequency: nHF) between resting and treatment conditions were performed by using a non-parametric test (Wilcoxon signed-rank test), and statistical significance was set at $p<0.05$.

A questionnaire was utilized for subjective evaluations. The subjects were requested to answer questions related to the psychological stress, Stress Response Scale (SRS-18), before the initiation of study procedure and after completing the study procedure of the treatment condition to compare subjectôs stress level before and after the study procedure of the treatment condition. Results of SRS-18 were examined using the paired *t*-test for statistical analysis. In addition, intensity of subjective pain sensation was evaluated using a visual analogue scale (VAS) on a scale of 0 to 10 before and after CSM treatment (pre- and post-CSM). Paired *t*-test was performed for statistical analysis on the results of VAS. Cervical muscle tone was measured bilaterally at the superior part of the trapezius muscle utilizing a Muscle Meter PEK 1 (Imoto Inc., Kyoto, Japan). Salivary amylase was also measured using an Amylase Monitor (Nipro Inc., Osaka, Japan) to examine changes in an autonomic function. The measurements of muscle tone and salivary amylase were performed pre- and post-20-minute resting, before FDG administration, and also before and after CSM, before FDG administration. The two-way analysis of variance (two-way

ANOVA) and the post-hoc test were performed on measurements of muscle tone and salivary amylase to determine differences in measurements between the resting and treatment conditions.

Chiropractic Spinal Manipulation (CSM)

Activator Methods (AM), one of chiropractic techniques, was utilized in the present study as a treating procedure and performed by a same chiropractic practitioner, a proficiency rated doctor of AM. The basic scan protocol in AM was performed on all subjects which includes examinations on seventh, fifth, second, first cervical vertebrae (C7, C5, C2, C1) and the occiput (Occ) in this order on the cervical spine[26)]. Instrumental spinal adjustments using an activator adjusting instrument (AAI) was performed in subjectsô prone position without their movements such as cervical rotation, lateral flexion, and extension if positive to the examination (Fig. 1,2). The examination includes tests in AM as well as static and motion palpation on the cervical area to find a joint dysfunction called subluxation. During the palpation, a tender nodule and a decreased joint motion were observed at an area of the joint dysfunction. Line of drive in the adjustment was toward the plane line of the facet; anterior superior internally on C7, C5 and C2, internally on C1, and anterior inferiorly on the occiput. Strength of the instrumental adjustment was set at 138.8+/-9.3 N in Fast Fourier Transform (FFT) peak magnitude with resonant frequency of 34.8+/-1.6 Hz, and duration of the adjustment was 1 to 5 ms (fig. 1).[27)]

FDG-PET Analysis

Brain PET images were analyzed to identify regional changes of glucose metabolic rate using a software package statistical parametric mapping (SPM2) [28) 29)], Functional Imaging Laboratory, London, UK. An FDG brain template distributed by Montreal Neurological Institute, McGill University, Canada[28)], was used for anatomical standardization (spatial normalization) of the PET images by applying Affine and non-linear transformations. The normalized data were smoothed using Gaussian kernel of 12 mm full width at half maximum (FWHM) to compensate for errors in spatial normalization. Voxel-wised paired *t*-test for statistical analysis was conducted based on the general linear model to find differences in the regional cerebral activity in the resting and treatment conditions. The height threshold for the significance was set at $p < 0.001$ without corrections for multiple comparisons, and extent threshold was set as 10 voxels minimum.

Correlation Analysis

A correlation analysis between regional cerebral glucose metabolism and intensity of subjective pain sensation, results of VAS, was performed utilizing SPM 2. These parameters were entered as covariates into the design matrix for each PET image. The height threshold for the significance was set at $p<0.001$, and extent threshold was set at 10 voxels minimum. Additionally, correlation analyses between values in volume of interest (VOI) on the FDG-PET analysis and values of subjective evaluations, VAS of pain intensity and SRS-18, were performed.

Frequency-domain analysis of HRV

HRV refers to variations between two consecutive heart beats which are assessed from R-R intervals, and the frequency of the peak R waves is taken for evaluating autonomic activity. There are two components in the HRV spectrum; low frequency (LF: 0.04 to 0.15 Hz) and high frequency (HF: 0.15 to 0.4 Hz) components accordingly. For HRV analysis, the values of spectral components (LF and HF) are obtained for evaluation of sympathetic and parasympathetic activities. HRV analytical software (University of Kuopio, Finland) was used for the evaluation. Consecutive heart beat intervals (R-R interval) were measured after determining peak QRS complex to obtain HRV spectral components (LF and HF). Power spectral analysis of heart rate variability in frequency domain was performed to determine autonomic sympathetic and parasympathetic parameters (LF and HF) with fast fourier transformation technique (FFT)[30]. LF and HF parameters were distinguished from frequency bands. The normalized values of LF and HF were determined for final elucidation of HRV which were expressed as normalized LF and HF (nLF and nHF). Hence, normalized

LF and HF (nLF and nHF) were measured according to following formulas: nLF = LF / (LF + HF) X 100 and nHF = HF / (LF + HF) X 100, respectively[31)].

RESULTS

FDG-PET analysis revealed significant changes in regional cerebral metabolism between resting and treatment conditions ($p<0.001$). Increased glucose metabolism was observed in the inferior prefrontal cortex (BA 47), anterior cingulate cortex (BA 32), and middle temporal gyrus (BA 21) whereas decreased glucose metabolism was observed in the cerebellar vermis and visual association cortex (BA 19) in the treatment condition (Table 1, Fig. 3).

Results of subjective measures revealed significant differences between the resting and treatment conditions. SRS-18 scores were significantly lower in the treatment condition (mean +/- S.D.: 5.2+/-5.3) than in the resting condition (10.4+/-8.3) ($p=0.003$) (Fig. 4). Comparisons on scores of subjective pain sensation (VAS) revealed significant differences between before (0.5~6.2; 3.6+/-1.7) and after CSM (0.1~3.2; 1.0+/-1.1) ($p<0.001$) (Fig. 4).

The adjusted areas in CSM included 1 subject on C7, 4 subjects on C5, 10 subjects on C2, 8 subjects on C1, and 4 subjects on Occ. The number of adjustment for each subject was 1 to 4 areas (2.3+/-1.2 areas) depended on the examinations. According to a survey to subjects of the present study one year after the experiments, duration of the relief of their cervical pain was 5 to 90 days (22.6+/-31.0 days). The pain rating at the time of the survey was 0 to 7

(2.4+/-2.3) on the scale of 0 to 10.

Measurement of cervical muscle tone also revealed significant difference between the resting and treatment conditions. Values of cervical muscle tone measured in bilateral shoulder regions before and after resting/treatment phases are shown here as the followings; the measures for the resting condition was 56.8+/-3.3 in the right side, 58.8+/-2.4 in the left side before resting phase and 56.3+/-3.7 in the right, 57.8+/-3.0 in the left after resting phase, respectively (Fig. 5). The measures for the treatment condition were 57.3+/-4.4 in the right, 58.8+/-3.7 in the left before the treatment phase and 51.1+/-4.8 in the right, 53.7+/-3.5 in the left after the treatment, respectively (Fig. 5). The result of two-way ANOVA was good, and the post-hoc test revealed significant differences bilaterally in the values of measurement after CSM compared to other muscle tone values (Table 2a,2b, Fig. 5).

Reduced salivary amylase values were observed on the subjects with chiropractic treatment. In the resting condition, the measurement values were 32.3+/-29.8 before resting and 43.1+/-36.9 after resting (Fig. 6a). The measurement values in the treatment condition were 27.0+/-19.2 before treatment and 19.5+/-12.4 after the treatment (Fig. 6a). Although two-way ANOVA indicated a significance on the alternate effect (conditions x measurements, p=0.04), any significant difference was not detected on the post-hoc test (Table 3, Fig. 6a). However, the differences between values of the pre- and post-measurements revealed barely significant difference between the conditions; -10.8+/-19.5 in the resting condition and

7.5+/-17.0 in the treatment condition ($p=0.04$) (Fig. 6b).

A correlation analysis revealed a positive correlation between VAS scores on the intensity of subjective pain sensation and the regional cerebral glucose metabolism in the orbitofrontal cortex (BA 11) and in the caudate nucleus ($p<0.001$) (Table 4) (Figure 7). Negative correlation to subjective pain sensation was not detected in any brain regions. A correlation analysis between values of VOI in the cerebellar vermis and VAS of the pain intensity indicated a positive correlation; coefficient of correlation was 0.59 ($p=0.002$). A positive correlation was also detected between values of VOI in the cerebellar vermis and scores of SRS-18; coefficient of correlation was 0.58 ($p=0.008$).

HRV analysis indicated differences in nLF and nHF between resting and treatment conditions. The value of nHF was increased (mean +/- SE: 45 +/- 5.3), and the value of nLF was relatively decreased (62 +/- 8.4) in the treatment condition compared to the resting condition (nHF: 35 +/- 3.6, nLF: 65 +/- 3.6) (Figure 8). However, statistical analysis on the values of nLF and nHF showed that the differences were not significant.

DISCUSSION

Researches on CSM have been performed worldwide, and its efficacy on musculoskeletal symptoms has been well documented. Previous studies also indicated a relationship of the vertebral dysfunction and the autonomic function[4) 16) 17) 18) 19) 20)]. Additionally, a functional neuroimaging study on CSM utilizing single photon emission computed tomography (SPECT) indicated decreased regional cerebral blood flow at the left cerebellum related to adverse reactions after manual cervical manipulation[25]. Although this study focused on the adverse reaction of CSM, the effects on the cerebral activity remain unknown and the clinical effects of CSM remain poorly understood. A recent chiropractic research documented the need to investigate the effects of spinal manipulation on cerebral activity whilst gaining a deeper understanding of the neurophysiological effects of CSM as it pertains to both musculoskeletal and non-musculoskeletal complaints[10]. Therefore, the present study may contribute to the chiropractic field for elucidating a part of neurophysiological effects on CSM.

In the present study, all subjects who had cervical pain at the time of the experiment were included since cervical pain is one of the symptoms commonly experienced by chiropractic patients. Also, one of the causes of the cervical pain is considered to be psychological stress. Researchers indicated that diagnosis and management of cervical pain should routinely include psychological stress[32] [33] [34] [35] [36] [37], and the psychological stress causes sympathetic activation[38] [39] [40]. Therefore, we believe that it is possible to compare the autonomic function in resting and treatment conditions in cervical pain patients. The usefulness of CSM for cervical pain is considered controversial due to adverse reactions of the cervical adjustment. Studies by University of California at Los Angeles (UCLA) reported adverse effects of cervical manipulation, commonly increased neck pain or stiffness and less frequently headache or radiating pain [41) 42)]. The investigator therefore selected AM as the treating procedure in the present study. AM is a form of research-based spinal manipulative therapy[26)] in which high-velocity, relatively low-force impact instruments, AAI, are used[26)]. Survey reports have estimated that the AAI are in use by more than half of all chiropractic practitioners in the U.S.[43) 44)]. In addition, investigations on AAI have been performed in light of safety concerns related to general cervical manipulation[26) 27)45)].

Deactivation of the cerebellar vermis

In the present study, the most significant change was detected in the cerebellar vermis, which was deactivated in the treatment condition compared to the resting condition. The cerebellar vermis is considered to be playing a role in pain perception. Neuroimaging studies revealed a pattern in the cerebellar activation during pain response[46) 47) 48)]. A study by Shiraishi et al. indicated glucose metabolic changes in the cerebellum in 13 of 18 patients suffering regional pain syndrome[49)], and many authors have indicated this similar activation in the cerebellum[50) 51)]. In the present study, all subjects had neck pain at the time of the experiment, and the result of VAS on the subjective pain intensity indicated significantly lower values after CSM. The correlation analysis also revealed a positive correlation between values of VOI in the cerebellar vermis and VAS of the pain intensity. Thus, deactivation of the cerebellar vermis may be related to pain reduction in the subjects. In addition, the cerebellar vermis is considered to be concerned with the autonomic nervous system. Previous studies have further suggested cerebellar involvement in the regulation of autonomic responses in aversive conditioning[43, 51, 52]. Removal of the cerebellum was shown to impair performance of autonomic functions such as salivary, cardiac, and respiratory conditioning[46, 51, 52]. Sacchetti et al. stated that these effects on aversive conditioning can be localized to the cerebellar vermis[46]. An early study documented that stimulation of the cerebellar vermis, not the cerebellar hemispheres, inhibited vasomotor tone which had been previously increased by peripheral stimulation[46, 53]. Thus, deactivation of the cerebellar vermis may be related to reduction of the sympathetic tone in the present study. The cerebellar vermis is also known to be concerned with mental stress. Ploghaus et al. found that painful heat activated the anterior cerebellum around the vermis while a sensory cue that anticipated the painful stimulation led to activation of the posterior cerebellar vermis[46, 47]. Mental stress causes sympathetic activation[37, 38, 39], and stress-related disorders are frequently accompanied by increased sympathetic activity as well as muscle tone[37]. Some studies have revealed that chronic activation of the sympathetic nervous system in chronic stress facilitates tonic and painful muscle contractions as it has been suggested for chronic tension-type headache and work-related myalgia[37, 54, 55]. In the present study, the measurement of muscle tone indicated significantly lower values after CSM at which point the cerebellar vermis was deactivated. The correlation analysis also revealed a positive correlation between values of VOI in the cerebellar vermis and scores of SRS-18. Therefore, we suggest that a result of the present study, the deactivation of the cerebellar vermis, may be preceded by reduction in sympathetic tone, muscle tone, and pain.

Activations in the anterior cingulate cortex and inferior prefrontal cortex

The anterior cingulate cortex and inferior prefrontal cortex were activated in the treatment condition in the present study. The cingulate cortex is known to be involved in generation of autonomic responses[37) 56) 57)]. A previous study indicated that performance of relaxation tasks elicited maximal activation in the anterior cingulate region[57)]. This region of the limbic cortex has been implicated in cognitive and emotional processing and as a part of the midline attentional system that involves the dorsolateral prefrontal cortex[58) 59) 60)]. The lateral prefrontal regions are deactivated during various cognitive tasks compared to the resting condition[61) 62) 63) 64) 65)]. In other words, it is possible that the lateral prefrontal regions are activated during relaxed condition. According to those previous studies, activation of the inferior prefrontal cortex in the treatment condition in the present study may indicate a relaxation effect of CSM. Thus, the results of the present study suggest that activation of the anterior cingulate cortex and inferior prefrontal cortex may arise from reduced sympathetic tone. An additional possibility of the increased glucose metabolism in the anterior cortex after CSM is pain perception in subjects caused by the instrumental treatment of CSM although it consists of a high-velocity and relatively low force adjustment which might be painful for some of the subjects. In fact, a recent fMRI study documented that the anterior cingulated cortex was activated by painful stimulation which is consistent with the recruitment of a thalamocortical mechanism that participates in the modulation of pain-related cortical responses[66].

Measurement of salivary amylase

The measurement of salivary amylase revealed decreased values after CSM and increased values after resting in the present study. Recently, salivary measures have become increasingly important in psychoneuroendocrinological research especially on stress[65]. One parameter of salivary measures thought to be reflecting stress-related changes in the body is the salivary enzyme alpha-amylase[67] [68] [69] [70] [71]. Authors have documented an increase in salivary amylase in people undergoing psychological stress[67] [68]. Therefore, it is possible that a decrease in salivary amylase is observed in people in a relaxed condition. Regarding the present results of PET analysis, it seems that the reduction in salivary amylase after CSM is related to the activated brain areas and deactivated areas which are related to reduction of the sympathetic tone. Additionally, it is thought that a result of salivary amylase measurement, increased values after resting and decreased values after the treatment, may be due to the present study protocol; subjects were directed to sit on a sofa with their eyes closed for 30 minutes before the PET scanning in the resting condition that might be irritable for some of them while they might get relaxed after CSM.

Correlation analysis

In the present study, a positive correlation was observed between the regional cerebral glucose metabolism and VAS scores of subjective pain sensation in the orbitofrontal cortex and in the caudate nucleus. Although the orbitofrontal cortex is among the least understood regions of the human brain, it is considered to be concerned with activity of the autonomic nervous system according to a number of previous studies[14) 58) 72) 73) 74) 75) 76) 77)]. Some previous studies documented that the orbitofrontal cortex has emerged as the strongest candidate for linking food and other types of reward to hedonic experience that could be associated with relaxed condition [14) 78)]. A recent study documented that being in a congruent emotional state, irrespective of the emotion, activates the orbitofrontal cortex[79)]. Tran et al. also indicated the orbitofrontal cortex was activated during pain durations concerned with the thalamocortical mechanism[66)]. Additionally, in a recent study, Otti et al. documented a positive correlation between the orbitofrontal cortex and the level of pain which one perceives when putting oneself into the painful situation[80)]. In the present study, all subjects had cervical pain, and the pain was relieved after CSM as indicated by the comparison on VAS scores of the pain intensity that might lead subjects in a congruent emotional state. Thus, the positive correlation between glucose metabolism in the orbitofrontal cortex and VAS scores of subjective pain sensation matches other results of the present study. The caudate nucleus is a part of the basal ganglia that works in the motor function, cognition, emotion, and the reward[81) 82)]. The caudate nucleus is also considered to be concerned with the autonomic nervous system as a part of the striatum which receives inputs form the thalamus and cerebral corticies[14)]. Previous studies indicated the involvement of the caudate nucleus in the process of visceral perceptions[14) 83)]. Additionally, researchers have documented that the caudate nucleus is related to increased motor activity in humans which causes chronic pain disorders such as tension-type headache and chronic low back pain[36) 84) 85) 86)]. The tension-type headache is mainly related to cervical pain that subjects had at the time of the experiment in the present study. Therefore, the positive correlation between glucose metabolism in the caudate nucleus and VAS scores of subjective pain sensation may directly support other results of the present study. Although putative functions of the orbitofrontal cortex and the caudate nucleus are directly related to other results of this study, those two areas have not detected in the FDG-PET comparison analysis. The discrepancy is thought to be caused by the height threshold of the correlation analysis ($p<0.001$). In the fact that a positive correlation was detected between the regional cerebral glucose metabolism in the cerebellar vermis (x,y,z: 4, -48, -6) and VAS scores of subjective pain sensation with the height threshold of $p<0.005$.

Heart Rate Variability analysis

In the present study, increased nHF and relatively decreased nLF were observed in HRV analysis. The heart rate is traditionally thought to be influenced by autonomic nervous system[4) 15)], and the heart rhythm electrocardiographic R-R interval has long been considered to be indicative of autonomic nervous activity[4) 15) 87)]. LF component mainly reflects both sympathetic and parasympathetic activities while HF component reflects parasympathetic activity[4) 15) 88)]. In a previous study on aroma therapy, Duan et al. indicated similar results on HRV analysis, increase in the HF component after lavender administration, and they concluded relaxation effect (increased parasympathetic activity) of the lavender aromatic treatment[15)]. Previous studies on the effects of CSM using HRV analysis also suggested that the spinal manipulative therapy may have an effect on the patientôs autonomic tone by modulation of the central control mechanisms[4) 88)]. The present study demonstrated changes of HRV (nLF and nHF) after CSM indicating tendency of increase in the parasympathetic activity (increased nHF) and relative decrease in the sympathetic activity (decreased nLF); however, statistical analysis did not indicate significant differences between resting and treatment conditions.

Summary

In summary, this is the first study that demonstrates the effects of CSM on regional cerebral metabolism by comparing resting condition and treatment condition utilizing FDG-PET. Brain areas concerned with pain perception, mental stress and the autonomic nervous system are deactivated in the treatment condition compared to the resting condition. Also, the activated areas in the treatment condition are considered to be concerned with the relaxation effect of CSM. Additionally, the correlation analysis between the regional cerebral glucose metabolism and subjective pain sensation demonstrated a positive link between the brain areas related to the autonomic nervous function and muscular pain perception. Therefore, it is possible to state that the present study demonstrated the effects of a single CSM treatment in the central nervous system in terms of regional cerebral metabolic changes concerned with relaxation effect, reduced muscle tone and pain reduction on the subjects. However, results of the present study was not reflected only CSM. During the experimental procedure, subjects experienced other factors such as conversations with examiners, physical contacts by examiners, and the administration of HDG which might cause changes in cerebral activities.

CONCLUSIONS

In the present study, medical evaluations on effects of a single CSM treatment were performed on the effects on the changes in the cerebral activities after CSM with comparison to the cerebral activities after resting by utilizing FDG-PET analysis. FDG-PET analyses indicated possible effects of a single CSM treatment in terms of metabolic changes in some brain regions which are associated with relaxation effect, reduced muscle tone and decreased pain intensity. Therefore, the present study indicated that a single CSM treatment potentially influences indirectly to the central nervous system. Further medical evaluations on the spinal manipulative therapy are needed to support the results of the present study since the number of the subject is relatively small in the present study. Additionally, further physiological studies on effects of the spinal manipulative therapy are needed not only for the chiropractic field but also for the field of alternative cares of medicine.

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Table1. Results of PET comparison analysis

	Anatomical Region	coordinates x,y,z (mm)			Brodmann Area (BA)	cluster	voxel Z score
Activation	IPC	54	24	-8	47	19	3.82
	MTG	-48	-36	0	21	30	3.73
	ACC	22	24	38	32	10	3.48
Deactivation	CV	4	-42	-18		121	4.62
	VAC	4	-90	24	19	46	3.64

The height threshold: p<0.001, extent threshold: 10 voxel minimum

Abbreviations: IPC=Inferior Prefrontal Cortex, MTG=Middle Temporal Gyrus, ACC=Anterior Cingulate Cortex, CV=Cerebellar Verrmis, VAC=Visual Association Cortex.

Table 2a. Results of two-way ANOVA on values of cervical muscle tone: R

Sources	Freedom	Mean Square	F value	P Value
Conditions	1	65.33	3.89	0.07
Pre, Post values	1	133.33	24.18	<0.001
Conditions x Measurements	1	96.33	11.95	0.005
Cut point	1	146965.33	4.85.81	<0.001

Abbreviations: ANOVA=Analysis of Variance, R=Right

Table 2b. Results of two-way ANOVA on values of cervical muscle tone: L

Sources	Freedom	Mean Square	F value	P Value
Conditions	1	52.08	6.00	0.03
Pre, Post values	1	114.08	36.46	<0.001
Conditions x Measurements	1	48.00	28.54	<0.001
Cut point	1	157323.00	5797.50	<0.001

Abbreviations: ANOVA=Analysis of Variance, L=Left

Table 3. Results of two-way ANOVA on values of salivaly amylase

Sources	Freedom	Mean Square	F value	P Value
Conditions	1	2494.08	3.60	0.08
Pre, Post values	1	33.33	0.21	0.66
Conditions x Measurements	1	1008.33	5.73	0.04
Cut point	1	44530.08	25.53	<0.001

Abbreviation: ANOVA=Analysis of Variance

Table 4. Results of correlation analysis

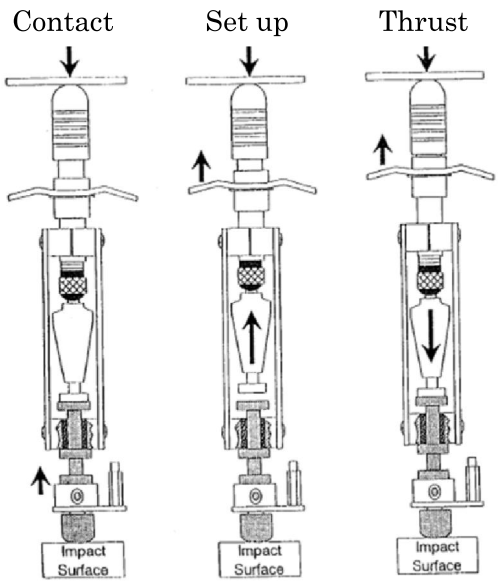
Brain area	Brodmann Area (BA)	Coordinates x,y,z (mm)	Cluster	t-value	z-score
Orbitofrontal cortex	11	18, 56, -22	23	6.81	3.81
Caudate nucleus		22, -20, 22	11	5.85	3.55

Brain areas which are positively correlated to scores of VAS on subjective pain sensation.

The height threshold: p<0.001, extent threshold: 10 voxel minimum

(a)

(b)



(c)

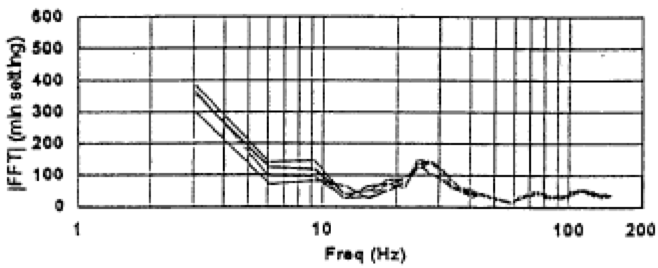


Figure 1. Activator Adjusting Instrument (AAI) and its characteristics

The figure shows an adjusting instrument which are utilized in the chiropractic treatment (a). AAI is a high-velocity, relatively low-force impact adjusting instrument (b), and (c) shows frequency characteristics of AAI input force for minimum force setting which was used in the present study. Fast Fourier Transform (FFT) spectrum frequency axis is log scale.

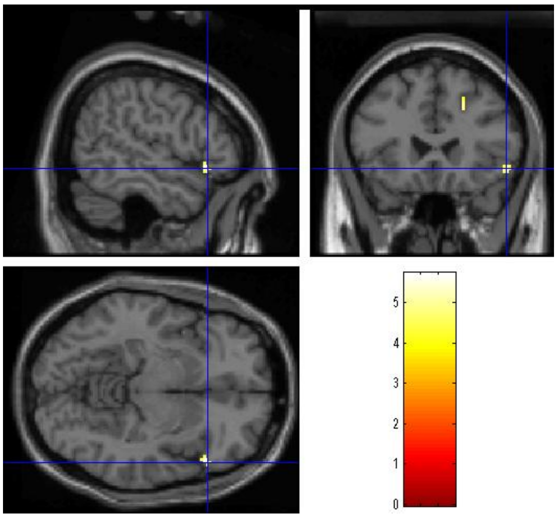
(a)

(b)

Figure 2. Instrumental treatment of the cervical spine

The figure shows a chiropractic adjustment on the atlas (C1); line of the drive in the adjustment is lateral to medial (a) and the axis (C2); line of drive in the adjustment is toward anterior, superior, and medial (b) using an activator adjusting instrument.

(a)



(b)

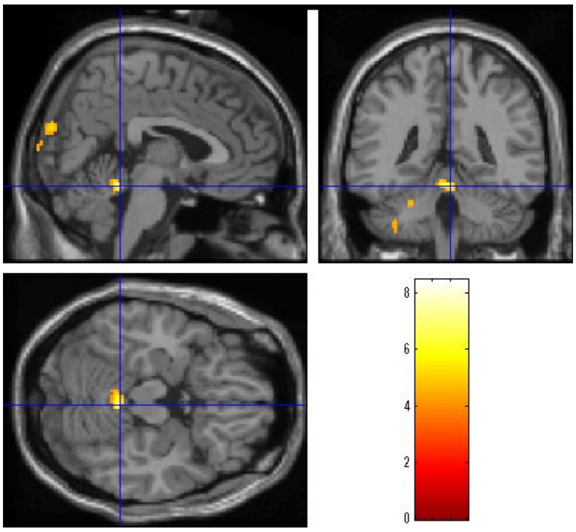


Figure 3. Regional activation (a) and deactivation (b) due to chiropractic treatment. Brain regions with metabolic increase in the treatment condition (a) and the regions with metabolic reduction in the treatment condition (b). Both figures demonstrate results of voxel-by-voxel comparison of regional brain glucose metabolic images using statistical parametric mapping (SPM) (height threshold: $p<0.001$, extent threshold: 10 voxel minimum).

(a) (b)

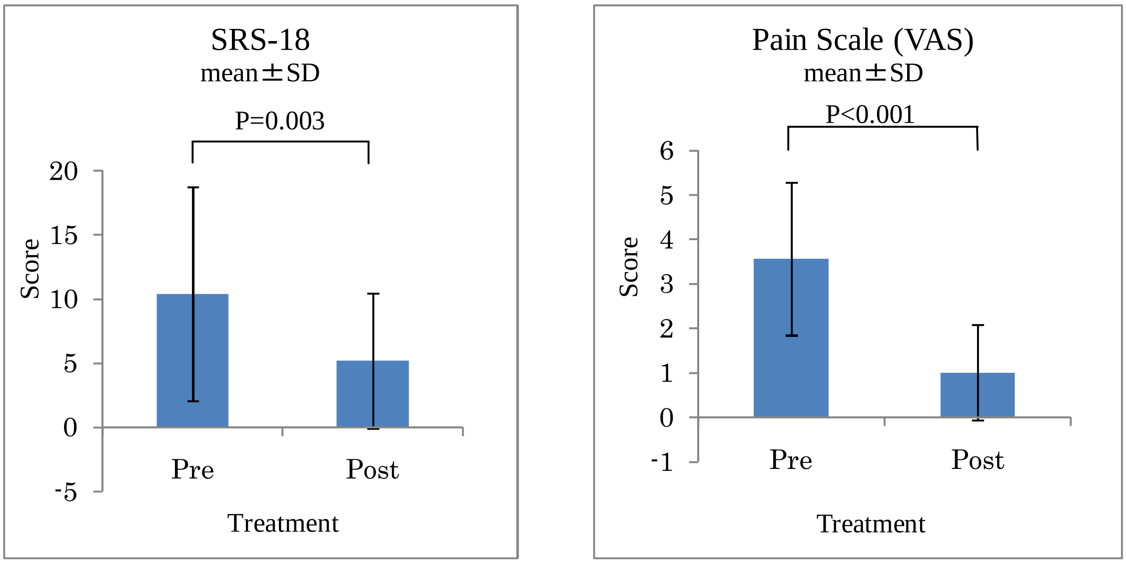
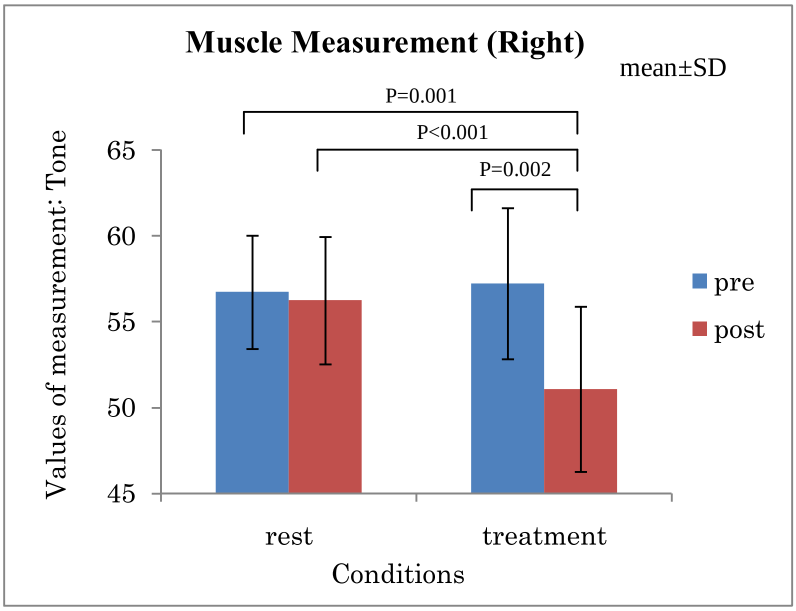


Figure 4. Results of subjective evaluations

Mean value of SRS-18 is lower after CSM (a). Mean value of Pain scale on VAS is lower after CSM (b). P values are indicated by a pared *t*-test for values of each scale. Error bars are described in standard deviation. Abbreviations: SRS-18=Stress Response Scale-18, VAS: visual analogue scale



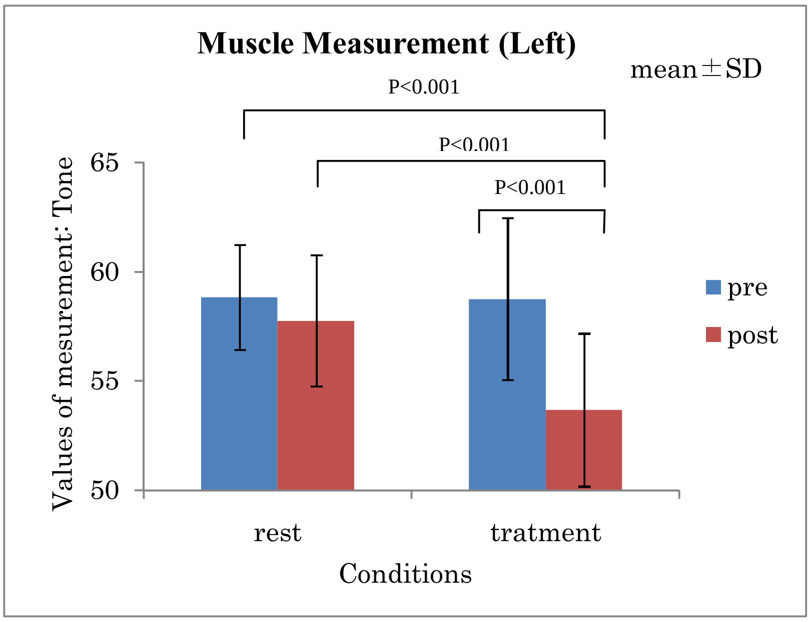
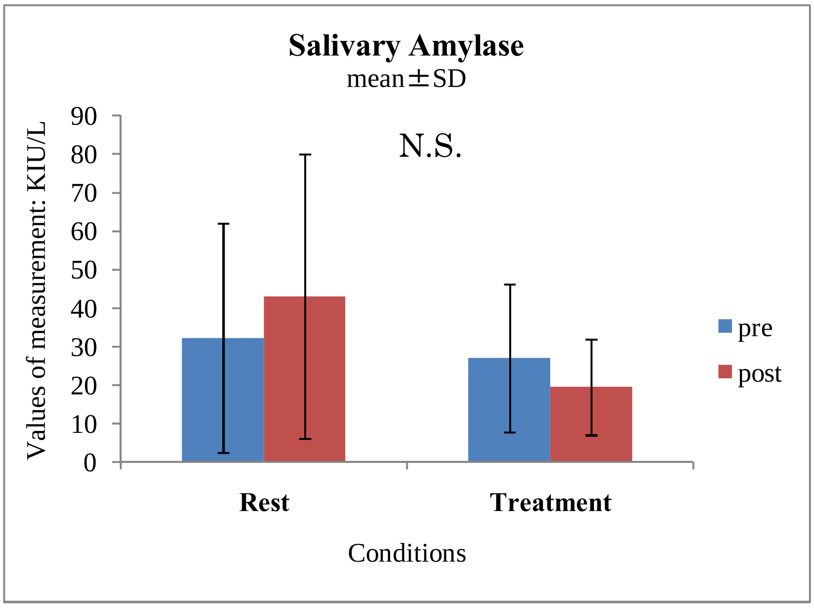


Figure 5. Results of measurements of muscle tone

The post-hoc test on measurements of the cervical muscle tone indicated significantly lower values bilaterally after the treatment compared to other measured values. Error bars are described in standard deviation.

(a)



(b)

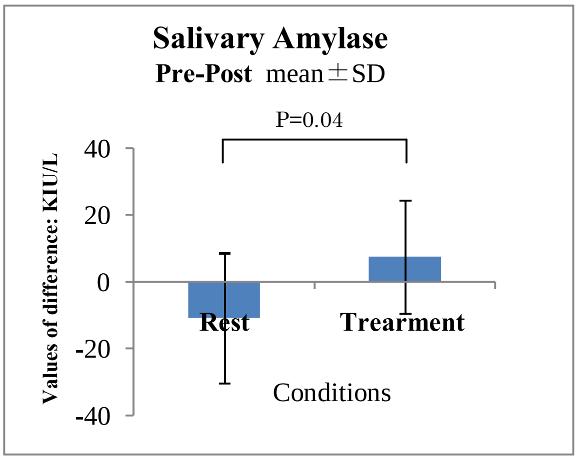


Figure 6. Results of salivary amylase measurements

Mean values of salivary amylase are relatively lower after chiropractic treatment while it is relatively higher after resting; however, the differences are not statistically significant (a). Although post-hoc test after the two-way analysis of variance did not indicate significant differences, a pared *t*-test on values of differences (pre - post) showed a barely significant difference between resting and treatment conditions (b). Error bars are described in standard deviation (SD). Abbreviation: N.S.=not significant

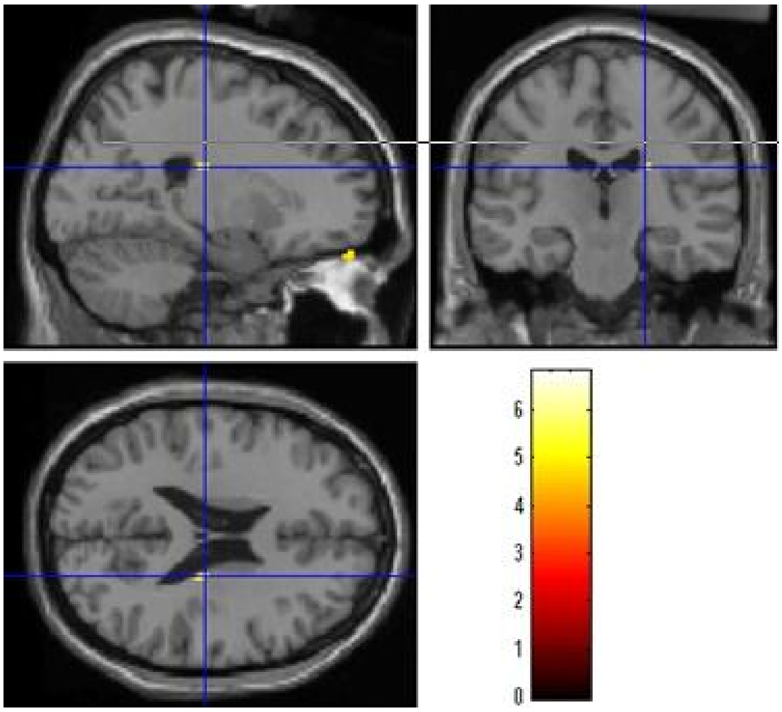


Figure 7. Results of correlation analysis.

Brain regions that are positively correlated with subjective pain sensation. The figure demonstrates results of voxel-by-voxel analysis of regional brain glucose metabolic images using statistical parametric mapping (SPM) (height threshold: p<0.001, extent threshold: 10 voxel minimum).

(a) (b)

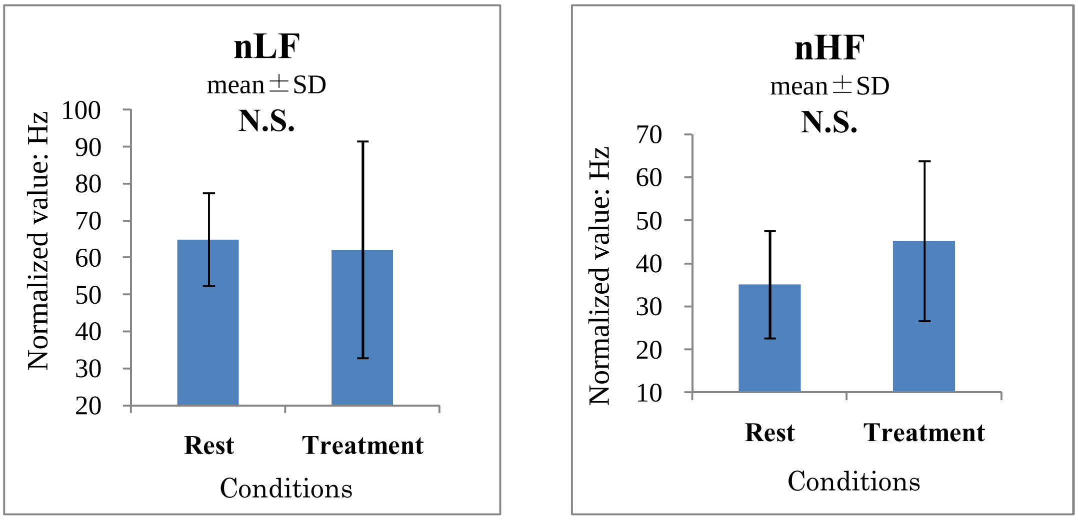


Figure 8. Results of heart rate variability (HRV) analysis.

HRV analysis showed relative increase in nHF indicating increased parasympathetic activity (a) and relative decrease in nLF indicating decreased sympathetic activity (b); however, the differences are not statistically significant. The data indicate mean value +/-standard deviation (SD)

Abbreviations: nHF=normalized high frequency, nLF=normalized low frequency, N.S.=not significant